PROTEIN COMPOSITION OF THE GLYOXYSOMAL MEMBRANE

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Received 31 July 1974

1. Introduction

Membranes of glyoxysomes, a special form of peroxisomes, have been recently isolated and characterized to some extent [1–3]. Knowledge of the protein composition of these membranes should be of interest, since, up to now, mainly because of the lack of availability of suitable preparations, the protein pattern of well-defined fractions of isolated biological membranes has been determined only in fairly few cases.

This paper describes the results of an SDS*-electrophoretic study on the protein composition of glyoxysomal membranes and on the solubilization of these membrane proteins by salt and detergents.

2. Methods

Glyoxysomes were isolated from the endosperm tissue of castor beans (*Ricinus communis* L. var *zanzibariensis*) as previously described [1]. Glyoxysomal membranes were prepared as described [1] from isolated glyoxysomes of high purity.

Isolated membranes were either tested directly by SDS electrophoresis or treated with salt and/or detergent before electrophoresis as described in 'Results'.

Electrophoresis was carried out according to Weber et al. [4] in 7.5% polyacrylamide gels either in tubes, or in slabs in the electrophoresis chamber described by Stegemann [5]. Gels were stained with Coomassie Brilliant Blue R 250. Phosphorylase *a*, serum albumin, catalase, pyruvate kinase, glutamate dehydrogenase, ovalbumin, aldolase, glyceraldehyde-3-phosphate dehydrogenase, lactate dehydrogenase and cytochrome *c* were used as molecular weight standards.

Protein was determined according to Lowry et al. [6] and values were corrected for interference by high sucrose concentrations [7].

3. Results

Different types of membrane preparations were subjected to SDS gel electrophoresis: 1) Membranes separated from soluble constituents of glyoxysomes by a mild osmotic shock procedure in 0.01 M Tricin, 0.001 M EDTA (pH 7.5) [1]. After isopycnic sucrose gradient centrifugation these 'untreated membranes' were diluted 1.5-fold with 0.05 M Tricin (pH 7.5), and pelleted by centrifugation for 1 hr at 100 000 *g* at 4°C. 2) Particles obtained by traetment of membranes with 1% sodium deoxycholate. Membrane pellets were suspended in 1% sodium deoxycholate in 0.05 M Tricin (pH 7.5) and kept at 4°C for 30 min. Particles were separated from the solubilized material by centrifugation at 100 000 *g* for 1 hr. 3) Membranes treated with 1 M KCl, or 0.1% or 0.5% sodium cholate solution (0.05 M Tricin, pH 7.5), or 0.5% sodium cholate plus 1 M KCl (weight ratio of protein to cholate 1:0.5 or 1:3, resp.). Traetment was for 30 min at 4°C. Fractions were subsequently centrifuged as described above and the resuspended pellet and the supernatant dialyzed against 0.05 M Tricin buffer.

Figs. 1 and 2 show the SDS-electrophoresis patterns obtained with the different preparations. Gels of untreated membranes show the presence of at least eleven bands. Of these, the protein with a subunit mol. wt. of 66 000 is by far the most prominent. Treatment of the

* *Abbreviation*: SDS: sodium dodecyl sulfate.

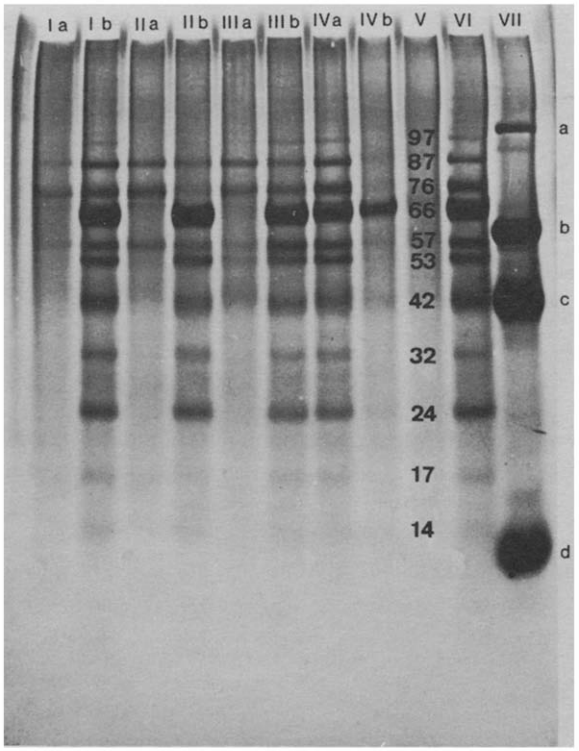

Fig. 1. SDS-polyacrylamide gel electrophoresis of glyoxysomal membrane proteins. I) supernatant (a) and pellet (b) after treatment with 0.5 fold amount of sodium cholate, II) supernatant (a) and pellet (b) after treatment with 3 fold amount of sodium cholate, III) supernatant (a) and pellet (b) after treatment with 1 M KCl, IV) supernatant (a) and pellet (b) after treatment with sodium cholate–KCl, V) molecular weights of membrane proteins ($\times 10^{-3}$), IV) untreated membranes, VII) molecular weight standards (a: phosphorylase *a*, b: catalase, c: ovalbumin, d: cytochrome *c*).

membranes with KCl or with sodium cholate are fairly ineffective in solubilizing membrane proteins. Surprisingly, with the exception of the 53 000 dalton band, the same bands are partly solubilized with detergent and with salt, which may indicate the presence of several unresolved bands of similar molecular weight. After treatment with sodium deoxycholate the major amount of the 66 000 dalton band remains particle-bound whereas all other main bands are solubilized extensively under such conditions. Combination of salt with a mild detergent, sodium cholate, is even more effective. By this treatment, a major part of the main polypeptide is also solubilized, but even here, a substantial amount of this protein remains particulate.

4. Discussion

The protein composition observed for glyoxysomal membranes is probably characteristic for peroxisomes, at least from plant material. It has been shown that mem-

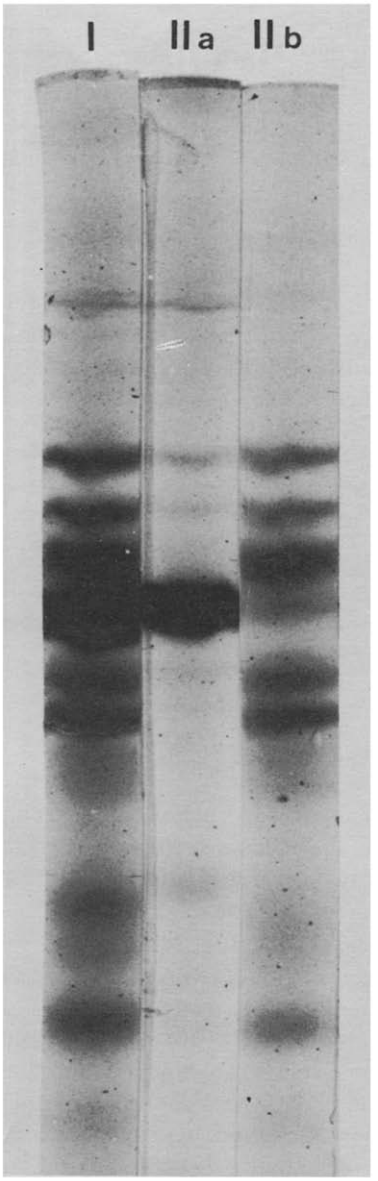

Fig. 2. Treatment of glyoxysomal membranes with sodium deoxycholate. I) untreated membranes, II) pellet (a) and supernatant (b) after treatment with deoxycholate.

branes of glyoxysomes from other seeds and of peroxisomes from greened cotyledons exhibit quite similar patterns (F. Fessl and H. Ruis, unpublished results).

The solubilization studies carried out indicate that most of the polypeptides in our membrane preparation are integral membrane proteins. Nevertheless, there is at least one clearcut difference in the behaviour of the various proteins. The main polypeptide seems to be integrated into the membrane much more strongly than other components. It seems very likely that this protein has an important structural function in peroxisomal membranes, which does, of course, not exclude the possibility that it also has enzymatic activity. It seems interesting that Hinman and Phillips [8] have obtained similar results with microsomal membranes from liver, where also one component is much more resistant to solubilization than others. The general protein pattern in glyoxysomal membranes is similar to that observed by Schnaitmann [9] for smooth or rough microsomal or outer mitochondrial membranes from liver. In all these cases most of the major protein components of the membranes have molecular weights between 50 000 and 70 000. That peroxisomal membranes are related to other components of the endomembrane system is also indicated by the fact that they contain an Antimycin A–insensitive NADH–cytochrome *c* reductase [1,10].

Acknowledgements

We thank Prof Dr O. Hoffmann-Ostenhof for his interest in and support of this work and Mr P. Zimniak for help with the experiments. A grant from the Fonds zur Förderung der wissenschaftlichen Forschung, Wien, Austria, is gratefully acknowledged.

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